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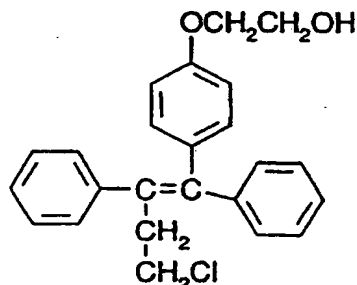
(51) International Patent Classification ⁶ : A61K 31/085	A1	(11) International Publication Number: WO 97/32574 (43) International Publication Date: 12 September 1997 (12.09.97)
<p>(21) International Application Number: PCT/FI97/00140</p> <p>(22) International Filing Date: 4 March 1997 (04.03.97)</p> <p>(30) Priority Data: 9604577.8 4 March 1996 (04.03.96) GB</p> <p>(71) Applicant (for all designated States except US): ORION-YHTYMÄ OY [FI/FI]; Orionintie 1, FIN-02200 Espoo (FI).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): HÄRKÖNEN, Pirkko [FI/FI]; Vuorelantie 3 B 43, FIN-20720 Turku (FI). MIETTINEN, Tatu [FI/FI]; Sateenkuja 3 C, FIN-02100 Espoo (FI). MÄNTYLÄ, Eero [FI/FI]; Kaistisentie 41 B, FIN-20760 Piispanristi (FI). KANGAS, Lauri [FI/FI]; Pasantie 3 B, FIN-21280 Raisio (FI). DeGREGORIO, Michael [US/US]; 7689 Shadow Oaks Lane, Granite Bay, CA 95746 (US).</p> <p>(74) Agent: ORION CORPORATION; Orion Pharma, Patent Service, P.O. Box 65, FIN-02101 Espoo (FI).</p>		<p>(81) Designated States: AM, AU, AZ, BA, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LK, LT, LV, MD, MK, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, UA, US, UZ, VN, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published With international search report.</p>
<p>(54) Title: SERUM CHOLESTEROL LOWERING AGENT</p> <p>(57) Abstract</p> <p>A method of lowering serum cholesterol levels comprising administering to a patient in need of such treatment an effective amount of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol, as well as pharmaceutical compositions useful in the method, is disclosed.</p>		

SERUM CHOLESTEROL LOWERING AGENT

This invention relates to the use of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol for lowering serum total or LDL cholesterol or increasing serum HDL cholesterol.

- 5 It has been demonstrated that elevated levels of serum cholesterol associated with low density lipoproteins (LDL) are a major contributing factor in the development and progression of atherosclerosis. Low serum HDL cholesterol is an independent risk factor for atherosclerosis. Therefore it is desirable to provide a method for reducing serum total cholesterol or LDL
10 cholesterol levels combined with an increasing effect on HDL cholesterol levels in patients with hypercholesterolemia or at risk of developing hypercholesterolemia.

- Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol, also known as (deaminohydroxy)toremifene, having the chemical structure represented by
15 formula,



is a metabolite of known antiestrogen drug toremifene. Toremifene is currently used clinically for the treatment of estrogen receptor positive breast cancer.

- 20 Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol has been earlier described e.g. in Sipilä, H. et al., "Metabolism of toremifene in the rat", J. Steroid Biochem., 36, 3, 211-215, (1990), in Kangas, L., "Biochemical and pharmacological effects of toremifene metabolites", Cancer Chemother. Pharmacol., (1990), 27, 8-12, in Anttila, M. et al., "Pharmacokinetics of
25 toremifene", J. Steroid Biochem., 36, 3, 249-252, (1990), in Simberg, N. et al, "In vitro and in vivo binding of toremifene and its metabolites in rat uterus", J. Steroid Biochem., 36, 3, 197-202, (1990), in Bishop, J. et al., "Phase I clinical

and pharmacokinetics study of high dose toremifene in postmenopausal patients with advanced breast cancer", Cancer Chemother. Pharmacol., 30, 174-178 (1992) and in the applicant's International Patent Application No. PCT/FI95/00475. It was shown that this compound is active in producing the
5 release of TGF- β but is devoid of significant hormonal activity.

Now it has been found that Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)-phenoxy]ethanol is active in lowering serum total and LDL cholesterol and increasing serum HDL cholesterol. Since this compound lack significant hormone associated side effects, it is especially suitable for lowering serum
10 total or LDL cholesterol or increasing serum HDL cholesterol, as well as in the treatment or prevention of atherosclerosis.

The invention provides a method of lowering serum total or LDL cholesterol levels or increasing serum HDL cholesterol levels, which method comprises administering to a patient in need of such treatment an effective
15 amount of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol.

The invention also provides a method for the prevention or treatment of atherosclerosis, which method comprises administering to a patient in need of such treatment an effective amount of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol.

20 The present invention also provides the use of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol in the manufacture of a medicament for use in lowering serum total or LDL cholesterol levels or increasing serum HDL cholesterol levels.

The invention also provides a pharmaceutical composition for use in
25 lowering serum total or LDL cholesterol levels or increasing serum HDL cholesterol levels comprising an effective amount of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol.

The invention also provides the use of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol in the manufacture of a medicament for the
30 prevention or treatment of atherosclerosis.

The compound of the invention may be administered in a variety of ways including orally, parenterally or transdermally using conventional forms of preparations, such as capsules, tablets, granules, powders, suppositories, injections, patches, suspensions and syrups. The term "effective amount"

means an amount of compound of the invention which is capable of lowering serum total or LDL cholesterol levels or increasing serum HDL cholesterol levels. The compound of the invention may be administered according to the method of the invention monthly, weekly or daily or several times a day
5 depending upon the patient's needs. A typical daily oral dosage is within the range of from about 0.5 mg to about 1000 mg, preferably from about 10 mg to about 800 mg, of the active compound. However, the dosage may be properly varied depending on the age, body weight and conditions of the patient as well as on the administration method. The compound of the invention may be
10 administered alone or together with other active compounds.

The compositions according to the invention can be prepared by the methods commonly employed in the art. In addition to the active compound the compositions may contain pharmaceutically acceptable additives commonly used in the art, such as carriers, binders, excipients, lubricants,
15 suspending agents and diluents. The amount of the active compound in the compositions of the invention is sufficient to produce the desired therapeutical effect, for example about 0.5 to 1000 mg, preferably about 10 mg to 800 mg, in unit dosage for both oral and parenteral administration.

The following examples illustrate the synthesis of the compound of the
20 invention.

EXAMPLES

Example 1.

Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol

(i) Z-4-[4-(2-benzyloxyethoxy)-phenyl]-3,4-diphenyl-but-3-en-1-ol

25 The reaction vessel was charged with toluene (790 ml), 48 % aqueous sodium hydroxide (790 ml), tetrabutylammonium bromide (2.12 g, 6.6 mmol) and Z-4-(4-hydroxy-1,2-diphenyl-but-1-enyl)-phenol (50 g, 0.16 mol) prepared by the method described by United States Patent 4,996,225. The mixture was refluxed for 30 minutes. Benzyl-(2-bromoethyl)ether (Grobelny D. et al.,
30 Tetrahedron Letters 28, 2639-42, 1979) (41.7 g, 0.19 mol) was added to the reaction mixture and the refluxing was continued for 2 hours. Then the mixture was cooled to room temperature, layers were separated and aqueous phase was washed with toluene. Toluene phases were combined, washed

with water, dried and evaporated to dryness. The residue was used in the next stage without further purification.

¹H NMR (300 MHz, CDCl₃): d 1.2 (1H, t, OH), 2.8 (2H, t, CH₂-C=), 3.6 (2H, dt, CH₂OH), 3.7 (2H, t, CH₂OBn), 4.0 (2H, t, CH₂OPh), 4.6 (2H, s, OCH₂Ph), 6.6 (2H, d, H-PhO), 6.8 (2H, d, H-PhO), 7.1-7.4 (15H, m, H-Ph).

(ii) Z-1-[4-(2-benzyloxyethoxy)-phenyl]-4-chloro-1,2-diphenyl-but-1-ene

Z-4-[4-(2-benzyloxyethoxy)-phenyl]-3,4-diphenyl-but-3-en-1-ol prepared in the previous stage was dissolved in acetonitrile (400 ml).
10 Triphenyl phosphine (103.5 g, 0.4 mol) and tetrachloromethane (120 g, 0.79 mol) were added and the mixture was refluxed for 2 hours. Then the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in methanol (160 ml) and water (40 ml) and extracted three times with petroleum ether (3 x 200 ml) at boiling point. Petroleum ether layers were
15 combined and evaporated to dryness under reduced pressure. The residue was crystallized twice from ethanol (700 ml). Yield 36 g.

¹H NMR (300 MHz, CDCl₃): d 2.9 (2H, t, CH₂-C=), 3.4 (2H, dt, CH₂Cl), 3.7 (2H, t, CH₂OBn), 4.0 (2H, t, CH₂OPh), 4.6 (2H, s, OCH₂Ph), 6.6 (2H, d, H-PhO), 6.8 (2H, d, H-PhO), 7.1-7.4 (15H, m, H-Ph).

20

(iii) Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)-phenoxy]-ethanol

Z-1-[4-(2-benzyloxyethoxy)-phenyl]-4-chloro-1,2-diphenyl-but-1-ene (36 g, 0.08 mol) was dissolved in the mixture of ethyl acetate (350 ml) and ethanol (350 ml). Palladium on carbon (5%, 0.28 g) was added and the
25 solution was flushed with hydrogen gas until there was not any starting compound left (thin layer chromatography). Palladium on carbon was filtered off through siliceous earth and the filtrate was evaporated. The residue was crystallized from the mixture of ethanol (155 ml) and water (65 ml). Yield 20 g.

¹H NMR (300 MHz, CDCl₃): d 2.9 (2H, t, CH₂-C=), 3.4 (2H, dt, CH₂Cl), 3.84-3.89 (2H, m, CH₂OH), 3.92-3.96 (2H, m, CH₂OPh), 6.6 (2H, d, H-PhO), 6.8 (2H, d, H-PhO), 7.1-7.4 (10H, m, H-Ph).

30

The effect of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol in lowering serum cholesterol levels is demonstrated by the following experiments.

EXPERIMENTS

In vivo study in rats

In this study adult female Sprague-Dawley rats were used. The compound of the invention was dissolved into vehicle (148 mM NaCl, 2.9% Magrocol 3000, 0.19% Tween 20) at different concentrations (0.5-10 mg/ml). The animals were dosed once daily with the solutions (1 ml/kg) by oral gavage for 28 days. The control animals received mere vehicle. The final daily doses of the compound of the invention were 0, 0.5, 1, 5 or 10 mg/kg. At the end of the treatment period blood samples were collected by heart puncture from anesthetized animals. The separated serum samples were analyzed for cholesterol, cholesterol precursor molecules and selected phytosterols by gas chromatography (Miettinen T., J. Lipid. Res. 29, 43-51, 1988).

The results are shown in Table 1. The test compound decreased serum total cholesterol level by 34% at the highest dose level. The relative phytosterol content also decreased slightly at the highest dose level (e.g. β -sitosterol level by 20%, Table 1). There was no significant changes in serum squalene or precursor sterol content, except in the relative lathosterol content that increased by about 50% (Table 1).

A preliminary data from a 3-month rat study supports the concept that Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol is a potent serum cholesterol lowering agent: daily dose of 0.5 or 2 mg/kg decreased serum total cholesterol level by 15-25 or 30-40 %, respectively.

TABLE 1. Effect on serum total cholesterol, lathosterol and β -sitosterol content in female rat after 4 weeks oral administration. The values (mean \pm SEM) are indicated as per cent of the control values

Dose (mg/kg)	Cholesterol	Lathosterol ^a	β -Sitosterol ^a
0.5	90.7 \pm 8.4	78.3 \pm 2.3	106.0 \pm 7.6
1	91.1 \pm 5.0	87.2 \pm 10.3	90.6 \pm 6.4
5	89.0 \pm 7.5	80.1 \pm 16.8	90.9 \pm 6.5
10	65.7 \pm 7.0*	146.6 \pm 14.7*	79.6 \pm 1.0*

^a Values relative to the cholesterol content

* = 2p<0.05

Cholesterol biosynthesis study

The effects on cholesterol biosynthesis were also studied *in vitro* in Hep G2 cell cultures using ^{14}C -acetate as cholesterol precursor. The test compound was added into the culture medium at concentrations from 0.001 to 5 micromolar. After 2 hours the culture was stopped and the newly synthesized cholesterol was quantitated by thin-layer chromatography.

In the *in vitro* cell culture system used the test compound did not inhibit cholesterol biosynthesis directly.

Phase I clinical study

In a phase I clinical double blind study the test compound was given perorally to healthy postmenopausal women daily for 12 weeks followed by a 2-weeks recovery period (10 subjects at each dose level). The doses used were 25, 50, 100 and 200 mg and serum lipide values were analyzed at 2 weeks intervals. Serum total cholesterol and HDL cholesterol content was determined by an enzymatic PAP-method, HDL cholesterol after PEG-precipitation. LDL-cholesterol values were calculated.

In the phase I clinical study at the dose level 200 mg the serum total cholesterol level decreased significantly ($p < 0.02$) from 2 weeks onwards and the maximal decrease was about 12% from the baseline. The decrease in serum LDL level was even more pronounced ($p < 0.003$): the decrease was maximally 22% from the baseline (dose level 200 mg). The test compound tended also increase HDL-cholesterol level, the increase was significant at the 200 mg dose level (an 10% increase over baseline, $p < 0.04$). The serum HDL/LDL cholesterol ratio tended to increase and this increase was highly significant ($p < 0.0001$) at the 200 mg dose level. During the recovery period the lipide values returned to the baseline level. No significant changes were seen in the placebo group.

Discussion

The mechanism by which the test compound decreases serum cholesterol content can be either direct inhibition of cholesterol biosynthesis, an effect on serum lipoprotein metabolism or an effect on cholesterol absorption from the gut, or some combination of these mechanisms. According to the present data a direct biosynthesis inhibition seems unlikely. A direct biosynthesis inhibition is not strong at the post-squalene stages of the

pathway as the accumulation of precursor sterols is minimal at the doses studied. The decrease in β -sitosterol level may indicate that cholesterol absorption is slightly impaired.

The human data suggests that Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol has beneficial lipid effects in clinical use: the cholesterol levels and especially harmful LDL-cholesterol level decrease markedly. In addition, an increase in the protective HDL-cholesterol level was noticed. In summary, the test compound is likely to inhibit or slow down the process of atherosclerosis in clinical use. The precise mechanism of action of the compound is to be elucidated with further experimentation.

CLAIMS

1. Use of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol in the manufacture of a medicament for use in lowering serum total or LDL
5 cholesterol levels or increasing serum HDL cholesterol levels.
2. A method of lowering serum total or LDL cholesterol levels or increasing serum HDL cholesterol levels, which method comprises administering to a patient in need of such treatment an effective amount of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol.
- 10 3. A pharmaceutical composition for use in lowering serum total or LDL cholesterol levels or increasing serum HDL cholesterol levels comprising an effective amount of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol.
4. A composition according to claim 3 which comprises a pharmaceutically acceptable carrier.
- 15 5. Use of Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)phenoxy]ethanol in the manufacture of a medicament for the prevention or treatment of atherosclerosis.

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/FI 97/00140

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/085

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CANCER CHEMOTHER. PHARMACOL., vol. 27, no. 1, 1990, pages 8-12, XP000675248 L. KANGAS: "Biochemical and pharmacological effects of toremifene metabolites." cited in the application see the whole document ---	1-5
Y	J. CLINICAL ONCOLOGY, vol. 13, no. 12, 1995, pages 2900-2905, XP000675382 H. GYLLING ET AL.: "Tamoxifen and toremifene lower serum cholesterol by inhibition of delta 8-cholestenol conversion to lathosterol in women with breast cancer." see the whole document -----	1-5

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

12 June 1997

Date of mailing of the international search report

27.06.97

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